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Antistress And Immunomodulatory Activity Of Aqueous Extract Of *Momordica charantia*

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ABSTRACT

The present study was undertaken to evaluate antistress and immunomodulatory activity of aqueous extract of *Momordica charantia (MC)*. Antistress activity was evaluated by measuring the swimming time in mice and cold immobilization induced stress for 10 days in rats, using *Withania somnifera* (100mg/kg) as reference standard. Immunomodulatory activity was evaluated by carbon clearance assay and percentage adhesion of neutrophils to nylon fibers using Levamisole as reference standard. The degree of protection was determined by measuring gastric ulceration, adrenal gland and spleen weights and by measuring levels of serum glucose, AST and ALT. Swiss albino mice of either sex were divided into 4 groups such as normal control, MC lower dose (450 mg/kg, p.o), MC higher dose (900 mg/kg, p.o) and standard group, treated with standard drug Levamisole (50 mg/kg, p.o). MC increased the swimming time in mice significantly ($P<0.001$) and the results are comparable to that of standard *Withania somnifera*. MC has also significantly ($P<0.001$) reversed the cold immobilization induced changes in glucose, AST, ALT, ulcer score, weight of adrenal gland and spleen. MC improves the phagocytic index in a dose dependent manner. MC at higher dose significantly ($P<0.001$) increased the percentage of adhesion of Neutrophils to nylon fibers when compared with the normal control animals. The results were comparable with that of standard drug levamisole. *Momordica charantia* has significant antistress, immunomodulatory activity.

KEYWORDS: Anti-stress, Cold immobilization, Immunomodulatory, *Momordica charantia*, Neutrophil adhesion, Swim endurance.

INTRODUCTION

Stress is a response to physical, chemical, biological and emotional changes, consisting of a pattern of metabolic and behavioral reactions that helps in strengthening the organism (1). If the stress is extreme, the homeostatic mechanisms of the organism become deficit and the survival of the organism is threatened. Under these conditions, stress triggers a wide range of the body changes called General Adaptation Syndrome (GAS). The stimuli, which produce GAS, are called the stressors and range from physical to psychological factors including cold, heat, infection, toxins, major personal disappointment etc (1). In the indigenous system of medicine, there are many herbal drugs and formulations recommended to enable one to withstand stress without altering the physiological functions of the body. This, drug induced state of resistance against aversive stimuli is termed as Adaptogenic activity, and the drugs named Adaptogens (2). Stress alters the equilibrium of various hormones which have a significant impact on the immune response in general. Stress and depression have been shown to affect immune system functioning, with both immunosuppression and immune activation. Stress alters the physiological homeostasis of the organism resulting in various endocrinal and visceral responses (3). Stress involves complex biochemical, neurological and immunological mechanism and plays a crucial role in the genesis/progression of a variety of

disease states such as psychiatric disorder like depression, anxiety, immunosuppression, endocrine disorder including diabetes mellitus, impotency and cognitive dysfunction. *Momordica charantia,* family Cucurbitaceae, is commonly known as bitter gourd or bitter melon in English and karela in Hindi. Role of *Momordica charantia* in diabetes is of paramount importance as this plant serves various purposes in these patients—lowers blood sugar, delays complications (nephropathy, neuropathy, gastroparesis and cataract, athereosclerosis) (4). Apart from antidiabetic activity it also has antiulcer activity (5). *Momordica charantia* seeds possess antimicrobial activity (6), antispermatogenic activity and androgenic activity (7). Leaves, fruits and roots are also used to treat fever. They have also been used in reproductive health as an abortifacient, birth control agent, or to treat painful menstruation and to facilitate childbirth (8). The present study is taken up to investigate the antistress, adaptogenic and Immunomodulatory activity of *Momordica charantia* fruits.

MATERIALS AND METHODS

Preparation of extract

Fresh karela or fruits of *Momordica charantia* were procured from local market. Fresh unripe fruits were sliced; pulp and seeds were removed and then mechanically squeezed. The juice obtained was dried in hot air oven below 60° C to get dried powder (9). The powder obtained was passed through sieve no. 40. The drug solution (9 mg/ml) was made using water as a vehicle.

Experimantal animals

Albino rats, Wistar strain, of the both sex (140-160 gm) and Swiss albino mice of either sex (22-30 gm), maintained under standard laboratory conditions (25°±2°C, relative humidity 50±15%, light and dark cycle of 12h) and fed with standard pellet diet and water *ad libitum*, were used for the present study. All the experimental protocols were approved by the Instititional Animal Ethics Committee.

Swim endurance test

The animals were divided into 4 groups where six animals in each group were used for the study. Group 1 treated as vehicle control, Group 2 and 3 were administered the *Momordica charantia* 450 mg/kg, p.o. and 900 mg/kg, p.o. respectively and group 4 with reference standard *Withania somnifera* (100 mg/kg, p.o) for 7 days. The dose for mice was calculated based on LD_{50} (91.9 mg/100gm) values of *Momordica charantia* (9). On the 8th day, the animals were allowed to swim till exhausted in separate cylindrical containers (bucket) of dimension 32 X 21 cm, filled with water to a height of 25cm. The end point is taken when the animals drown and swimming time for each animal was noted (2).

Cold immobilization test

Albino rats, Wistar strain, were divided into 5 groups, consisting of six animals each. Group 1 treated as vehicle control, Group 2 as stress control, group 3 and 4 were treated with *Momordica charantia* 315 mg/kg, p.o. and 630 mg/kg, p.o. respectively and group 5 with reference standard *Withania somnifera* (100 mg/kg, p.o) for 10 days. The dose for rat was calculated based on LD_{50} values of *Momordica charantia* (9). After 2 hours, the stress was induced by making the animals immobilize in plastic boxes. The boxes with the animals were then placed in a fridge (temp. 4° C) for 2 hours daily. The animals were then released and placed back into their respective cages. The procedure was followed for 10 days continuously. The animals of all the five groups were then fasted from the 9th day onwards and on the 10th day; blood was collected from retro-orbital sinus and analyzed for glucose, AST and ALT using semi auto analyzer. Glucose was estimated by End point, Enzymatic GOD-POD method where as AST and ALT was estimated by Kinetic method, using SPAN diagnostic kits (Code No.LG071 and LG021 respectively). The animals were sacrificed and weights of adrenal gland, spleen and ulcer score were calculated (10). The stomach was opened by cutting through greater curvature and degree of ulceration was examined with a hand lens. The scoring system followed is normal colored stomach-0, red coloration-0.5, spot ulcers-1, hemorrhagic stresks-1.5, ulcers three to five-2, ulcers more than five-3.

Carbon clearance assay

Male Swiss albino mice (25–30 gm) were divided into four groups, consisting of six animals each.

Group 01, 02, 03, 04 were treated with vehicle, *Momordica charantia* (450 mg/kg, p.o), *Momordica charantia* (900 mg/kg, p.o) and Standard drug levamisole (50 mg/ kg, p.o) for 5 days. Forty eight hours after the last treatment, mice were injected via the tail vein, carbon ink suspension (0.1 ml). Blood samples were drawn (in EDTA solution 5 µl) from the retro-orbital sinus at 0 and 15 min., a 25 µl sample was mixed with 2 ml of 0.1% sodium carbonate solution and its absorbance was determined at 660 nm (11).

The phagocytic index was calculated using the following equation:

Phagocytic index = ^{k}(sample)/ ^{k}(control)

Where $K = (Log_e OD_1 - Log_e OD_2) / 15$

OD_1= optical densities at 0 minutes and,

OD_2= optical densities at 15 minutes, respectively.

Neutrophil Adhesion test

Male Swiss albino mice of weighing 22–25 gm were divided into 4 groups of 6 animals each. Group 01, 02, 03, 04 were treated with vehicle, *Momordica charantia* (450 mg/kg, p.o), *Momordica charantia* (900 mg/kg, p.o) and Standard drug levamisole (50 mg/ kg, p.o) for 14 days. On the 14th day of drug treatment, blood samples were collected by puncturing the retro-orbital plexus into heparanized vial and analyzed for total leucocyte counts (TLC) and differential leucocyte counts. Differential leucocytes counts (DLC) by fixing blood smears and staining with Field stain I and II- Leishman's stain. After initial counts, blood samples were incubated with 80 mg/ ml of nylon fibers for 15 min. at 37 ° C. The incubated blood samples were again analyzed for TLC and DLC. The product of TLC and percentage Neutrophil gives Neutrophil index (NI) of blood sample. Percent neutrophil adhesion was calculated as shown below (12).

$$\text{Neutrophil adhesion } (\%) = \frac{NI_u - NI_t}{NI_u} \times 100$$

Where, NI_u = Neutrophil index of untreated blood sample.

NI_t = Neutrophil index of treated blood sample.

Statistical analysis

The results were expressed as Mean ± SEM and analysis was carried out by one-way ANOVA. Post-hock analysis was done by Tukey's multiple comparison tests to estimate the significance of difference between various individual groups.

RESULTS

As shown in table 1, there was a significant (P<0.001) increase in the swimming time with lower and higher doses of *Momordica charantia*, which were comparable with the results due to pretreatment with standard drug *Withania somnifera*. As seen from table 2, in stressed animals there was a significant (P<0.001) increase in ulcer score, glucose, AST, ALT levels, weight of adrenal gland and significant (P>0.001) decrease in weight of spleen when compared with normal control rats. *Momordica charantia* significantly produced dose dependent inhibition of increase in gastric ulcer score, glucose, AST, ALT levels, weight of adrenal gland and decrease in spleen weight. The results are comparable to that of reference standard *Withania somnifera*.

Table 1: Effect of aqueous extract of Momordica charantia on swimming time in Mice.

Group	Treatment	Dose (mg/kg, p.o.)	Swimming Time (min.)
01	Normal control	——	227.70 ± 2.69
02	Momordica charantia	450 mg/kg	341.16 ± 4.12*
03	Momordica charantia	900 mg/kg	470.00 ± 4.05*
04	Withania somnifera	100 mg/kg	545.00 ± 3.77*

All values are given in mean ± SEM, n = 6. One way ANOVA followed by Tukey's multiple comparison test.
*P<0.001 Vs Normal control.

As indicated in fig fig 1, *Momordica charantia* significantly increases the phagocytic index in a dose dependent manner. *Momordica charantia* at higher dose significantly (P<0.001) increased the percentage of adhesion of neutrophils to nylon fibers when compared with the normal control animals (fig. 2). The results were comparable with that of standard drug levamisole.

DISSCUSSION

Animals when forced to swim in a restricted space become immobile after an initial period of vigorous activity. This immobility signifies behavioral despair, resembling a state of mental depression (2). *Momordica charantia* significantly

Table 2: Effect of aqueous extract of Momordica charantia on ulcer score, weights of adrenal gland, spleen and glucose, AST and ALT levels in rats.

			Weights of organs		Biochemical estimations		
Group	Treatment	Ulcer Score	Adrenal gland (mg/100gm)	Spleen (mg/100gm)	Glucose (mg/dl)	AST (IU/L)	ALT (IU/L)
1	Normal control	0 ± 0	30.83 ± 0.945	208.83 ± 3.79	87.41 ± 1.434	82.46 ± 1.461	76.83 ± 2.187
2	Stress control	3.08 ± 0.153+	66 ± 1.291+	130 ± 2.864+	130.16 ± 1.293+	135.58 ± 2.856+	135.6 ± 0.993+
3	*Momordica charantia* (315 mg/ kg) p.o.	2 ± 0.129*	51.83 ± 1.352*	151.83 ± 1.905*	118.63 ± 1.234*	121.25 ± 1.276*	114.3 ± 1.538*
4	*Momordica charantia* (630 mg/ kg) p.o.	1.41 ± 0.327*	45.33 ± 0.881*	182 ± 2.436*	102.36 ± 1.105*	102.03 ± 2.162*	97.86 ± 1.119*
5	Reference standard *Withania somnifera* (100 mg/ kg) p.o.	1.25 ± 0.214*	40.73 ± 0.679*	187.33 ± 3.343*	97.15 ± 1.357*	91.65 ± 1.187*	88.23 ± 1.151*

All values are given in mean ± SEM, n = 6. One way ANOVA followed by Tukey's multiple comparison test.
+P< 0.001 Vs normal control,
*P< 0.001 Vs stress control

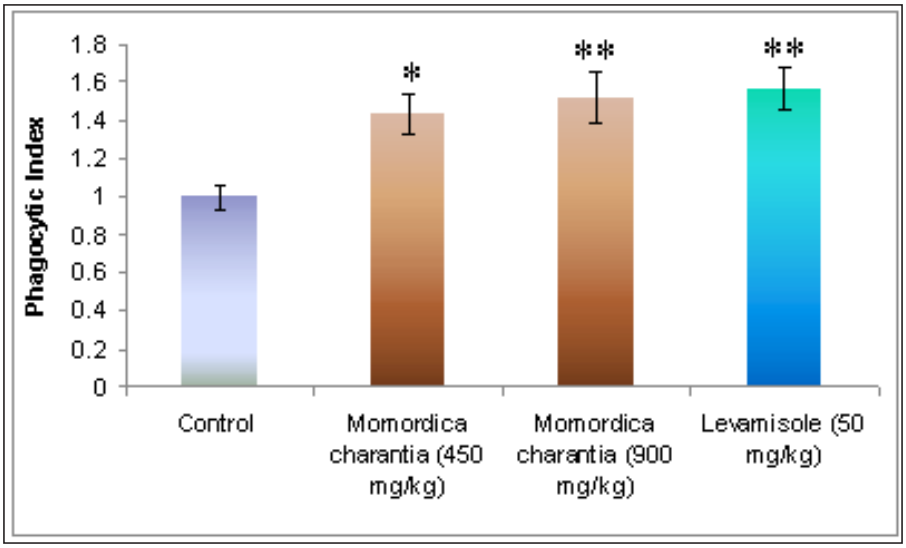

Figure 1: Effect of the *Momordica charantia* on phagocytic index in mice
All values are given in mean ± SEM, n=6; One way ANOVA followed by Tukey's multiple comparison test.**P<0.01, *P< 0.05 Vs control

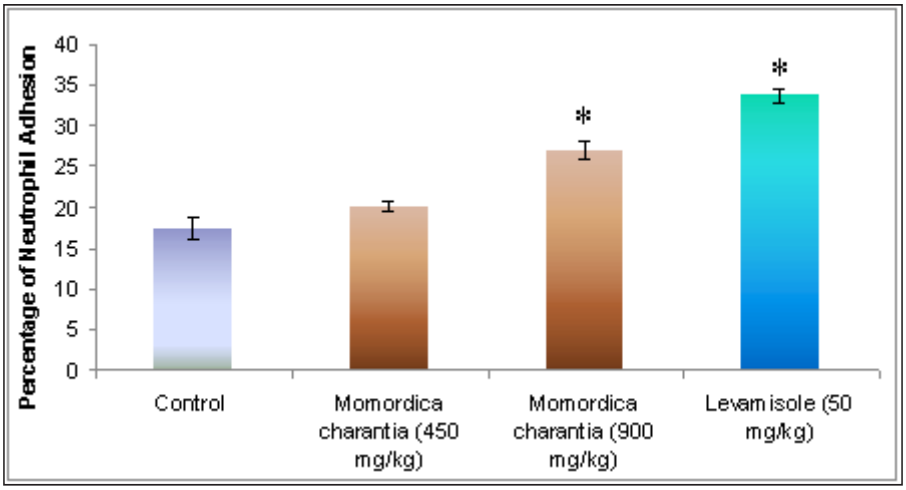

Figure 2: Effect of the *Momordica charantia* on percent neutrophil adhesion in mice
All values are given in mean ± SEM, n=6; One way ANOVA followed by Tukey's multiple comparison test. *P< 0.001 Vs control.

increased swimming time and has enhanced the physical performance longer than untreated (control) group confirming their adaptogenic nature.

In present study, the significant increase in blood glucose level was observed because, under stressful conditions adrenal cortex secretes cortisol in man and corticosterone in rats. Hyper secretion of cortisol helps in maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis (13). Pretreatment with the *Momordica charantia* as well as reference standard drug *Withania somnifera* significantly (P<0.001) reduced the elevated glucose levels indicating their suppressant effect on hyper activity of adrenal cortex and maintained the homeostatic mechanism.

The marked increase in serum AST, ALT levels in stress induced animals is due to stimulation of hypothalamopitutary axis (HPA) and sympathetic system, resulting in, liberation of catecholamines and glucocorticosteroids, which inhibits the immune system at multiple sites like liver, kidney (14). *Momordica charantia* as well as reference standard drug *Withania somnifera* significantly (P<0.001) reduced the elevated serum AST and ALT levels, which may be due to inhibition of stimulation of sympathetic nervous system.

When the animals were subjected to stress, induction of gastric ulcers were observed. This is found to be due to the involvement and hyper activation of Para Ventricular Nucleus (PVN), which in turn stimulates the paracrine system. This causes the release of histamine, there by leading to increased secretion of acids (15). *Momordica charantia* as well as reference standard *Withania somnifera* significantly reduced (P<0.001) the stress induced gastric ulcers. This may be due to inhibition of hyper activation of Para Ventricular Nucleus (PVN).

The increase in weight of adrenals in stressed animals is due to the stress induced adrenomedullary response leading to increased production of corticotropic hormone that leads to increase in weight of adrenals (13). *Momordica charantia* and *Withania somnifera* has significantly (P<0.001) reduced the adrenal gland weight, this may be due to the reversal of the stress induced adrenomedullary response and hence decreased production of corticotropic hormone.

The decrease in weight of spleen may be due to recruitment of lymphocytes to blood from spleen which results in squeezing of the spleen (10). The pretreatment with the *Momordica charantia* and reference standard *Withania somnifera* significantly (P<0.001) increased the spleen weight. This may be due to inhibition of recruitment of lymphocytes to blood from spleen.

A variety of biological activities including adaptogenic activity were reported with flavionoids, tannins and phenolic glycosides (13). *Momordica charantia* contains biologically active chemicals that include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids (4). The adaptogenic activity may be due to these constituents where as standard drug *Withania somnifera* an established adaptogenic drug too contains glycosides, steroids and flvoniods (13).

Immunomodulatory agents of plant origin enhance the immune responsiveness of an organism against a pathogen by activating the immune system. Macrophages have a major role in modulating the immune system. Phagocytosis of pathogens by macrophages initiates the immune responses, which in turn modulates the adaptive response. The primary target of most of the immunomodulatory compound is believed to be macrophages, which play a key role in the generation of immune response.

Immune system plays vital role in stress. The Reticuloendothelial system (RES) is a multi-organ system whose primary function is phagocytosis. The rate of removal of colloidal carbon by intravascular phagocytes in the liver and spleen is a measure of the reticuloendothelial phagocytic activity (11). In carbon clearance assay, the *Momordica charantia* has showed a dose dependent significant rise in carbon clearance, which is comparable with the reference standard drug levamisole, indicating stimulation of reticuloendothelial system. When reticuloendothelial system is stimulated, there is increase in number of phagocytic cells, which engulf antigens, indicating increase in immunity.

Neutrophil plays an important role in enhancing immunity of the body against microbial infection by, chemotaxis, phagocytosis, exocytosis, and both intracellular and extra cellular killing (11). *Momordica charantia* 900 mg/kg p.o. significantly (P<0.001) increased the adhesion of neutrophils to nylon fibers. The results are similar and comparable with that of reference standard levamisole. Further studies are required to exactly elucidate the mechanism of immunostimulatory activity.

CONCLUSION

The aqueous extract of *Momordica charantia* possesses antistress and Immunomodulatory activity.

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